PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT : **LINK, Jr., Charles J., et al.**
SERIAL NO : 10/682,178
FILED : October 9, 2003
TITLE : ANTITUMOR VACCINATION USING ALLOGENEIC TUMOR CELLS EXPRESSING ALPHA (1,3)-GALACTOSYLTRANSFERASE

Grp./A.U. : 1643
Examiner : HOLLERAN, Anne L.
Conf. No. : 7316
Docket No. : P05859US01

DECLARATION OF GABRIELA R. ROSSI UNDER 37 C.F.R. § 1.132

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Gabriela Rita Rossi, hereby, declare:

1. I am a Senior Scientist working at the Tumor Immunology Section, at NewLink Genetics Corporation of Ames, IA and a co-inventor of the above-identified application.

2. I received a PhD in The National University of Córdoba (NUC), Argentina, in 1997. My research focuses on understanding how to inhibit tumor growth using cells expressing α(1,3)Galactosyl transferase epitopes.

3. A copy of my CV is attached herewith (Exhibit A, 6 pages).

4. I have read the Office Action mailed March 1, 2007.

5. This Declaration is submitted herein to demonstrate that one skilled in the art would be able to make the cell lines in the claims using the teachings of the Specification and

techniques well-known prior to Applicant's filing date. Therefore, producing these cells lines is not outside the realm of routine experimentation and is not unpredictable.

6. As disclosed and evidenced herein, this declaration presents results of experiments performed from December 5th, 2002 to December 28th, 2002. The commercially available cell lines of MCF7, A549, and BT20 were transformed with pLNCKG (the sequence of which is provided in Figure 2 of the application) to make the cell lines of HAB1, HAL1 and HAB2 using standard transformation and cell culture techniques. The transformed cells' expression of αGal was determined using fluorescence-activated cell sorter (FACS) analysis. The results of FACS demonstrate that production of cell lines of HAB1, HAL1 and HAB2 can be reproduced and yield a population of cells which have similar staining intensity or expression of αGal in two independent generation events. A report summarizing these results is attached and labeled as exhibit B.

7. The results indicate that the cell lines in the claims can be reproduced by one of ordinary skill in the art using routine techniques known at the time of filing. The specification provides adequate detail and direction for one skilled in the art to allow them to prepare the cell lines in the claims.

8. Although only data for reproducing the cell lines of HAB1, HAL1 and HAB2 are shown in Exhibit B, all techniques for making these cell lines are routine and do not constitute undue experimentation, thus allowing one skilled in the art to reproduce the cell lines of HAL1, HAL 2, HAL3, HAB1, HAB2, HAPA1, HAPA2, HAPA3, HAPA4, HAO1, HAO2, HAO3, HAO4, HAM1, HAM2, HAM3, HAC1, HAC2, HAC3, HAC4, HAC5, HAC6, HAC7, HAC8, HAC9, HAC10, HAC11, HAC12, HAPR1, HAPR2, HAPR3, or HAPR4.

9. I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Dated this 29th day of MARCh, 2007

EXHIBIT A

GABRIELA R. ROSSI

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Ames. Iowa 50010
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EDUCATION

1998 – 2003	Post Doctoral Fellow. Holland Laboratories. American Red Cross. Rockville Maryland National Institutes of Health (NIH). Bethesda Maryland Iowa Cancer Research Foundation. Des Moines. Iowa.
1992 – 1997	Ph.D. in Chemical Sciences. Immunology and Infectious Diseases. Chemical Sciences Faculty National University of Córdoba (NUC) Argentina
1986 – 1991.	Graduated in Clinical Biochemistry. Faculty of Chemical Sciences National University of Córdoba (NUC) Argentina.

CURRENT POSITION

September 2003- Present
Senior Scientist. Tumor Immunology Section. NewLink Genetics Corporation. Volunteer Position Iowa Cancer Research Foundation.

CURRENT RESEARCH PROJECT

Development of a Cancer Vaccine using the α(1,3)galactosyl epitope.
Main goal: Modeling the immune-mechanism of action in murine models of cancer.

WORK EXPERIENCE

Research

January 2002 – September 2003
Postdoctoral Fellow. Dr Charles Link's laboratory. Iowa Cancer Research Institute (Former Human Gene Therapy Research institute). Tumor Immunology Section. Iowa Methodist Medical Center. Des Moines Iowa
Project: Development of a Cancer Vaccine using the α(1,3)galactosyl xenoepitope. Modeling the mechanism of action in a murine model of cancer.

August 2001- December 2001.
Post Doctoral Fellow. Dr Steve Rosenberg's Laboratory. Tumor Immunology Section, Surgery Branch, National Cancer Institute, NIH. Bethesda MD
Project: Engineering murine T cells using lentiviral vector mediated gene transfer to express human recombinant IL-2 for treatment of experimental murine melanoma.

November 2000 – July 2001.
Post Doctoral Fellow. Dr. Richard Morgan's Laboratory. Clinical Gene Therapy Branch, National Human Genome Research Institute, NIH. Bethesda MD
Project: Lentiviral vector mediated gene transfer into murine lymphoid and myeloid cells for vaccination and/or immunotolerance induction in murine models.

December 1998 – October 2000.
Post Doctoral Fellow. Dr. Dorothea Scandella's Laboratory. Immunology Department, Holland Laboratory, American Red Cross. Rockville MD
Project: Induction of immune tolerance in FVIII-deficient knockout mice by co-stimulatory blockade.

July 1992 – May 1997.
Graduate Student. Dr. Diana Masih's laboratory, Immunology Department, NUC. Cordoba, Argentina
Project: Mechanism of immune suppression induced during experimental *Cryptococcus neoformans* infection in rats.

Clinical

1989 – 1991. National Clinical Hospital, NUC, Argentina. Laboratory sections of immunology, parasitology, mycology hematology, haemostasia, chemistry.

Teaching

October 2004- Present.
Supervisor of Dana Awwad, Master Student at Iowa State University, Genetics program. Project: Effect of IL-15 in the anti-tumor immunity induced by vaccines expressing aGal epitopes.

October 2004- October 2006.
Supervisor of Hannah Johnson. Undergraduate student. Iowa State University.

Summer 2002.
Supervised Two Undergraduate students.
Colin Holtze and Thad Rathe: Project: Development of the Serum Activity Microassay for anti-alpha gal antibodies determination in murine and human samples

1991 – 1997.
Undergraduate instructor in Parasitology, Mycology and Microbiology.
Department of Clinical Biochemistry, Faculty of Chemical Sciences, NUC, Argentina

1989 – 1991.
Undergraduate Instructor of the Introductory Course to Chemical Sciences.
Faculty of Chemical Sciences, NUC, Argentina

Other work experiences

2006	Peer Reviewer for Cancer Immunology Immunotherapy Journal.
2005- Present	Manager Animal Program, NewLink Genetics Corp.
2003	Member Institutional Anima Care and use Committee (IACUC). Iowa Cancer Research Foundation.
2002	Grant revision, Israel Cancer Research Fund. Workshop.
1994-1995	State Government Inspector of Food Quality Control in Cordoba Public Schools.

PUBLICATIONS

1. Toxicology and efficacy studies of allogeneic murine vaccines expressing α(1,3)galactosyl epitopes. **Rossi GR.** Mautino Mario R., Leyukole H, Koenigsfeld M, Vahanian Nicholas, Link CJ. *Manuscript in preparation.*
2. . High-throughput fluorescent screening of transgenic animals: phenotyping and haplotyping Hellrung, D. J., **G. Rossi**, and C. J. Link. *Cytometry A 69:1092*, 2006
3. Effective Treatment of Preexisting Melanoma with Whole Cell Vaccines Expressing a(1,3)-Galactosyl Epitopes **. Rossi, G.R.** Mautino, Mario R. Unfer, R. C. Seregina, Tatiana M. Vahanian, Nicholas Link, C. J. *Cancer Res, 65: 10555-10561, 2005.*
4. Complete protection against melanoma in absence of autoimmune depigmentation after rejection of alphaGal expressing melanoma cells.. **Rossi GR,** RC. Unfer, T Seregina and Charles J. Link. *Cancer Immunol Immunother, 54: 999-1009, 2005.*
5. High-efficiency lentiviral vector mediated gene transfer into murine macrophages and activated splenic B lymphocytes. **Rossi GR**, Mautino MR and Morgan RA. *Human Gene Therapy. 14(4):385-392, 2003.*
6. Long-term induction of immune tolerance after blockade of CD40-CD40L interaction in a mouse model of Hemophilia A. **Rossi GR**, Sarkar J. and Scandella D. *Blood, 97(9):2750-2757, 2001.*
7. Potential role for excretory-secretory forms of glutathione-S-transferase (GST) in *Fasciola hepatica*. Cervi LA, **Rossi GR** and Masih DT. *Parasitology. 119:627-33, 1999.*
8. Involvement of nitric oxide in protecting mechanism during experimental cryptococcosis. **Rossi GR**, Cervi LA, Garcia MM, Chiapello LS, Sastre DA., Masih DT. *Clin Immunol, 90(2):256-65, 1999.*
9. Lack of involvement of nitric oxide in the macrophage-mediate inhibition of spleen cells proliferation during experimental Cryptococcosis. **Rossi GR**, Cervi LA, Sastre DA, Masih D.T. *Clin. Immunol. Immunopathol. 86: 16-26, 1998.*
10. *Fasciola hepatica*-induced immunosuppression of spleen mononuclear cells proliferation. Role of nitric oxide. Cervi L, **Rossi GR**, Cejas H, Masih DT. *Clin. Immunol. Immunopathol. 87:145-154, 1998.*
11. Role of macrophages in the systemic resistance and in the immunomodulation during experimental *Cryptococcus neoformans* infection. *Rossi GR. Ph.D. Thesis. Faculty of Chemical Sciences, NUC, 1997.*
12. Biochemical basis for the killing of *Cryptococcus neoformans* by rat peritoneal cells. **Rossi GR**, Sastre DA, Rubinstein HR, Masih DT. *J. Med. Vet. Mycology 32, 405-414, 1994.*

SCIENTIFIC MEETINGS PRESENTATIONS (FIRST AUTHOR PAPERS)

1. ADD AACR poster presentation 2006 Washington DC.
2. Effective treatment of pre-established B16 melanoma tumors with a novel vaccine expressing a(1,3)Gal epitopes requires anti-aGal Antibodies. Gabriela R. Rossi, Mario R. Mautino, Tatiana Seregina, Charles J. Link. Basic Aspect of Tumor Immunology. Keystone Symposium. Keystone Colorado. March 2005.

3. Treatment of pre-established Subcutaneous Tumor and Pulmonary Melanoma metastasis with a novel vaccine expressing a(1,3)Galactosyl xenoepitopes. Annual Meeting American Association of Immunologist (AAI). Washington DC April 2004. Selected for Oral Presentation in Block Symposium Cancer Vaccines and Immunotherapies.

4. Treatment of Pre-established Subcutaneous Tumor and Pulmonary Melanoma Metastasis with a Novel Vaccine Expressing α(1,3)Galactosyl Xenoepitopes. 2004 Annual Meeting of the American Society of Clinical Oncology. June 5 - 8, 2004 in New Orleans.

5. αGalactosyl Transferase knockout mice survived lethal challenge with melanoma cells expressing αgal epitope and develop CTL against native αgal negative melanoma tumors. Annual Meeting of American Association Cancer Research (AACR), Washington DC. 2003

6. Protection against B16 challenge after vaccination with novel αGal melanoma vaccine in the α(1,3)galactosiltransferase (αGT) knockout mice model. Annual Meeting American Association of Clinical Oncologist (ASCO). Chicago. IL. 2003.

7. Treatment of Subcutaneous Melanoma After Vaccination with Cells Expressing Xenoepitopes in the α(1,3)Galactosiltransferase (αGT) Knockout Mouse Model. Annual Meeting of the American Society of Gene Therapy (ASGT). Washington DC 2003.

8. Long-term induction of immune tolerance after CD40-CD40L blockade in a mouse model of Hemophilia A. America Association of Immunologist (AAI), Seattle. 2000.

9. Experimental *Cryptococcus neoformans* infection: Role of Nitric Oxide. IIX Meeting of the Microbiology Argentinian Association. Argentina, 1998.

10. Biochemical basis for the suppressive proliferative response of lymphocytes during experimental rat Cryptococcosis. XLV Scientific Meeting of the National Society of Immunology. Argentina, 1997.

11. Histopathological interpretations of pulmonary lesions in experimental Cryptococcosis. XVI Scientific and Cultural Meeting of Pathologist of Córdoba. Argentina, 1996.

12. Lymphocyte Proliferation to LPS is modified during *Cryptococcus neoformans* infection. IV Latin American Congress of Immunology, XI Mexican Congress of Immunology. Mexico, 1996.

13. Lymphocyte Proliferation to Con A is decreased during *Cryptococcus neoformans* infection. IV Latin American Congress of Immunology, XI Mexican Congress of Immunology. Mexico, 1996.

14. Peritoneal cells activation is modified during *Cryptococcus neoformans* infection. IV Latin American Congress of Immunology, XI Mexican Congress of Immunology. Mexico, 1996.

15. Relationship between the clinical diagnosis and the mycological laboratory in oral Candidiosis. XXVIII Annual Meeting of the International. Association For Dental Research. Argentina, 1995.

16. Susceptibility of *Cryptococcus neoformans* to oxygen-derived compounds. VI National Congress of Mycology. XVI National Meeting of Mycology. Argentina, 1993.

17. Reactive oxygen intermediates production in experimental cryptococcosis. XL Scientific Meeting of the National Society of Immunology. Argentina, 1993.

18. *Fasciola hepatica* excretion-secretion products: effects on the immune response of rats immunized with the total antigen. Scientific Meeting of the Biologist Society of Cordoba. Argentina, 1991.

19. Statistic correlation between electrocardiografic alterations and anti-*T.cruzi* antibodies in a chagasic population from north Argentina. Scientific Meeting of the Biologist Society of Cordoba. Argentina, 1991.

MAJOR FIELDS OF TRAINING

Cell culture

Primary T and B lymphocyte isolation, purification and culture from rodent organs and human peripheral blood. Polyclonal stimulation of B cells for activation and proliferation assays.
Antigen-specific and polyclonal T cell activation and proliferation assays ($[H]^3$-thymidine incorporation and CFSE staining).
Macrophages/monocytes dendritic cells isolation purification and culture. *In vivo* and *in vitro* peritoneal macrophages activation.
Cytokine determination in lymphocyte cultures.
Maintenance of hybridoma cell lines and cryopreservation.
Microbiologic techniques (culture and typification of bacteria and fungus).

Techniques

Nitric oxide (NO) and reactive oxygen intermediates (ROI) determination in macrophages culture supernatants.
ELISA, immunofluorescence, immunohistochemistry.
Monoclonal antibodies production and purification.
Protein analysis and purification, radiolabeling of proteins.
Bethesda assay, APTT, anti-FVIII-antibodies determination by immune precipitation.
Molecular biology techniques (cloning, PCR, plasmid extraction and analysis, Western and Southern blotting).
FACS analysis (immune phenotype, apoptosis, activation, intracellular cytokines, etc).

Gene Transfer

Stable and transient transfections of mammalian cells.
HIV-based lentiviral vectors production, concentration and quantification.
Transduction of mammalian cells lines and mouse cells (lymphocytes and macrophages-dendritic cells) using retroviral vectors.

Animal handling

Mice: Subcutaneous and intravenous cancer cell inoculation, growth curves and survival. Immunization studies using CFA. Antibody production and cell mediated immune response evaluation (delayed type hypersensitivity tests, DTH). Breeding, backcrossing, genotyping. tail clipping Retro-orbital eye bleeding under anesthesia. Extraction and processing cells from peritoneal cavity, spleen, lymph node, thymus and bone marrow. IV, SC, IP injections. Intra craneal inoculation of fungal infective particle, subculture from brain tissue.
Rats: IP, IV SC injections. Immunizations with/without adjuvant, cells harvest from peritoneum and spleen.
Hamsters: Heart bleeding
Rabbits: SC immunization with adjuvant, ear vein bleeding
Mice strains: α(1,3)galactosy transferase knockout, CD1-knockout , CD1-α(1,3)galactosy transferase double knockout, FVIII-knockout (Hemophilic), CD40L knockout, CD40 knockout. RAG, SCID and nudes.

Mycologic and Parasitologic diagnosis
Extraction and processing of patient's samples (skin, nails, hair, genital fluids, respiratory and digestive tract fluids, cephaloraquid fluid, feces). Yeast isolation, culture and characterization.

HONORS, FELLOWSHIPS AND OTHER MERITS.

2003. Cooperative Research and Development Agreement (CRADA). NewLink Genetics Corporation.

2002. National Research Service Award, NIH, Postdoctoral Fellowship Application.

1997. Doctoral Thesis received "Astounding" qualification.

1995 – 1997. Doctoral Fellowship for Senior Training in Research. National Research Council of Argentina (CONICET).

1995. Bi-annual Outstanding Teaching Award. Category Teacher Assistant

1993 – 1995. Doctoral Fellowship for Initial Training in Research National Research Council of Argentina (CONICET).

1993 – 1995. Doctoral Fellowship for Training in Research. Selected State Research Council (CONICOR), Cordoba Argentina.

EXHIBIT B

Experiments to reproduce cell lines HAB1, HAL1 and HAB2 were performed from date December 5th, 2002 and December 28th, 2002. We summarize these experiments here to provide evidence that the cell lines of the claims indeed are reproducible following the description and methods set forth in the specification.

The retroviral vector pLNCKG, used to transduce the parental cell lines (see Table I of the specification) from which these cell lines are derived, was prepared by following the protocol described in Example 1 of the present application. The HYPERACUTE® human tumor vaccines were produced following the manufacturing protocol described in Example 12 of the present application. The same methods and compositions described in the specification were used to generate all cell lines.

As shown in the Declaration, Applicants have produced cell lines with exactly the same characteristics as the cell lines in the claims of 34-65 and 68-74. The vaccine cell compositions of claims 34-65 and 68-74 are all derived from cell lines that are commercially available from ATCC, for example, MCF7 is available under ATCC catalog no. HTB-22, A549 is available under ATCC catalog no. CCL-185, and BT20 is available under ATCC catalog no. HTB-19. Table 1 of the specification indicates the identity of the cell lines used to prepare each one of the vaccine cell compositions of claims 34-65 and 68-74 and the ATCC catalog number. Example 1 of the specification gives detailed description of how to produce retroviral supernatants from the alpha—(1,3)-galactosyltransferase encoding vector pLNCKG. Example 2 gives detailed protocols about how to transduce a cell line with retroviral vector pLNCKG. Example 12 gives detailed description of how to perform transduction of the listed human cell lines to generate HYPER ACUTE® vaccines. Example 12 also describes the anti-αGal antibody and its commercial source used to stain transduced cells for FACS sorting. The remainder of Example 12 describes how to generate master cell banks, working cell banks and production lots from these cell lines. Therefore, a person of skill in the art would be able to reproduce the compositions of the present invention without undue experimentation.

Figure 1A-1C and 1D-1F show the FACS analysis, performed on two different days, showing results of two independent transductions performed on cell line MCF7 with two separate pLNCKG vector preparations to generate the master cell banks for HAB1. Figs 1B and 1E show equivalent results for αGal in the unsorted cell population. After αGal staining with the 0-2605 anti-αGal antibody and FACS sorting and analysis, the vaccine preparations have the same level of αGal staining. The comparison of similar staining patterns, and similar proportion of $\alpha Gal^{(+)}$ cells before and after sorting, indicates that the method is reproducible when performed as described. Minor variations in the absolute values of fluorescence between the duplicate cell lines are due to minor differences in the calibration of the FACS machine on different days.

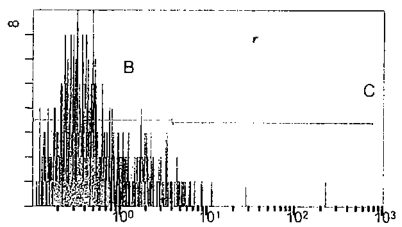
Figure 2A shows the anti-αGal staining for naïve A549 (Fig 1A) and Figs 2B and 2C show the analysis after the independent generation of two separate master cell banks (HAL1-MCB1 and HAL1-MCB2). These figures demonstrate that the procedure for making cell lines HAL1-MCB1 and HAL1-MCB2 is reproducible.

A third example is provided for the generation of HAB2. Figure 3A-3C show the analysis of two independently generated populations of transduced cell line BT20 with two different pLNCKG vector preparations. Figures 3B and 3C show the transduced population before FACS sorting. The intensity of αGal staining (which is one of the potency readouts to certify clinical vaccine preparations) is comparable for both batches of transduced cells.

These data indicate that the cell lines HAB1, HAL1 and HAB2 can be produced from commercially available cell lines following the teachings in the specification.

A

[A] PMT3 Log



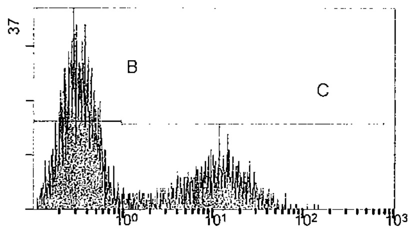
MCF7 negative control 2002-12-13.LMD

[A] PMT3 Log

Region	Number	%Total	%Gated	X-Mean
B	711	20.29	97.13	0.7
C	19	0.54	2.60	18.9

B

[A] PMT3 Log



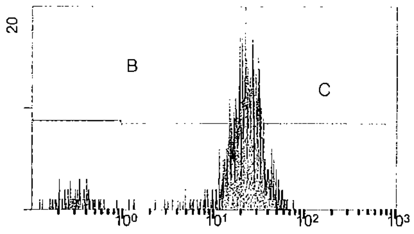
MCF7 CKg2 before sorting 2002-12-13.LMD

[A] PMT3 Log

Region	Number	%Total	%Gated	X-Mean
B	2897	28.97	65.44	0.4
C	1524	15.24	34.43	13.5

C

[A] PMT3 Log



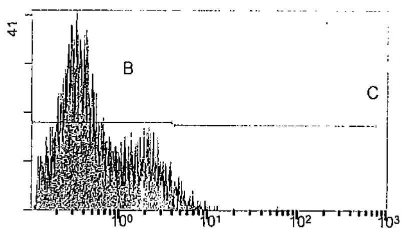
MCF7 CKg2 after sorting 2002-12-13.LMD

[A] PMT3 Log

Region	Number	%Total	%Gated	X-Mean
B	73	3.55	5.90	0.3
C	1165	56.66	94.10	24.7

D

[A] PMT3 Log



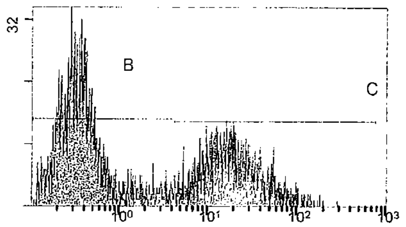
MCF7 Negative control 2002-12-12.LMD

[A] PMT3 Log

Region	Number	%Total	%Gated	X-Mean
B	5449	54.49	96.77	0.8
C	167	1.67	2.97	6.0

E

(F1)[A] MCF7 CKg1 before sorting 2002-12-12.LMD : PMT3 Log



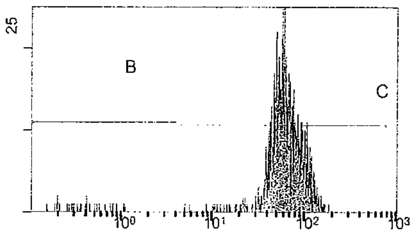
MCF7 CKg1 before sorting 2002-12-12.LMD

(F1)[A] MCF7 CKg1 before sorting 2002-12-12.LMD : PMT3 Log

Region	Number	%Total	%Gated	X-Mean
B	2925	29.25	60.92	0.6
C	1858	18.58	38.70	25.0

F

[A] PMT3 Log



MCF7 CKg1 after sorting 2002-12-12.LMD

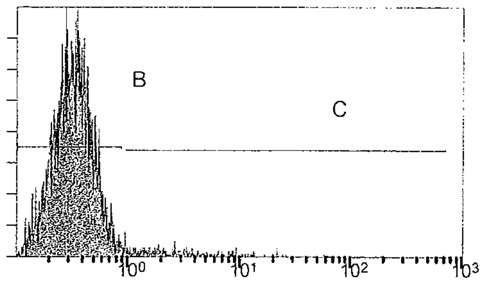
[A] PMT3 Log

Region	Number	%Total	%Gated	X-Mean
B	34	1.65	2.41	0.4
C	1375	66.65	97.59	68.2

Figure 1

[A] PMT3 Log

A



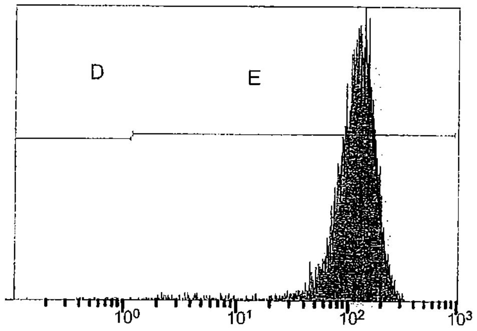
A549 Negative control 2002-12-11.LMD

[A] PMT3 Log

Region	Number	%Total	%Gated	X-Mean
B	6671	66.71	95.49	0.3
C	285	2.85	4.08	4.4

[A AND B AND C] PMT3 Log

B



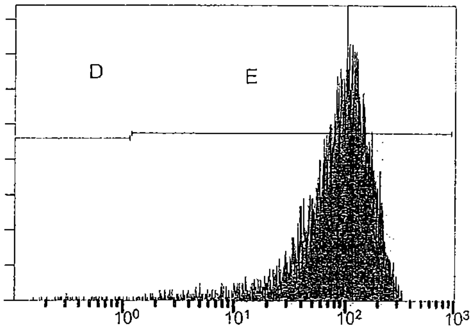
HAL1 MCB1 2002-12-28.LMD

[A AND B AND C] PMT3 Log

Region Number %Gated X-Mean

[A AND B AND C] PMT3 Log

C



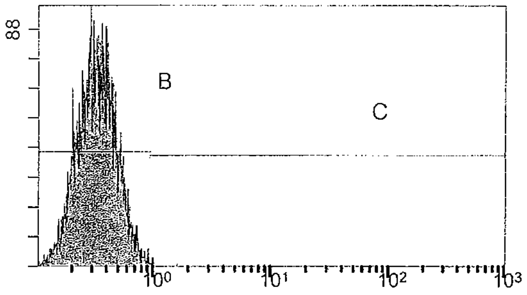
HAL1-MCB2 2002-12-26.LMD

[A AND B AND C] PMT3 Log

Region	Number	%Gated	X-Mean
D	39	0.41	0.5
E	9368	99.59	103.7

Figure 2

A

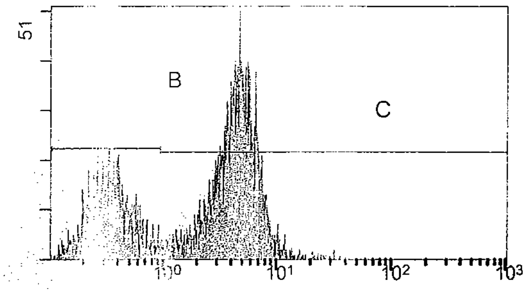

[A] PMT3 Log

BT 20 Negative control 2002-12-11.LMD

[A] PMT3 Log

Region	Number	%Gated	X-Mean
B	7320	99.78	0.4
C	14	0.19	1.0

B

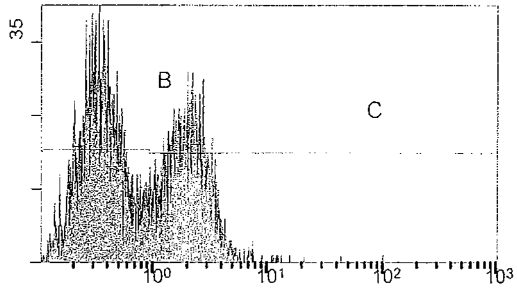


BT20 293Z.CKg1 before sorting 2002-12-10.LMD

[A] PMT3 Log

Region	Number	%Gated	X-Mean
B	1790	32.00	0.4
C	3803	68.00	4.8

C


[A] PMT3 Log

BT20 293.ZCKG2 before sorting 2002-12-11.LMD

[A] PMT3 Log

Region	Number	%Gated	X-Mean
B	3120	57.92	0.4
C	2274	42.21	2.3

Figure 3